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DATE: Tuesday, April 12, 2005

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☐	L3	L1 and helicobacter	2
☐	L4	hp30 or hp-30 or hp1588 or hp-1588	121
☐	L5	L4 and helicobacter	7
☐	L6	(29 or 29.5 or 30 or 30.5 or 31).clm. same (kd or kda or k-da or daltons or kilodaltons or kilo-dalton or mw or rmw or r-mw or size or weight or sds or page or western).clm.	92562
☐	L7	L6 same pylori	12
☐	L8	L6 same helicobacter.clm.	13
☐	L9	L8 not l5	10

END OF SEARCH HISTORY

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L5: Entry 4 of 7 File: USPT Jan 4, 2005

US-PAT-NO: 6838089
DOCUMENT-IDENTIFIER: US 6838089 B1

TITLE: Antigen delivery system and method of production

DATE-ISSUED: January 4, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Carlsson; Hans	Molndal			SE
Larsson; Anette	Olofstorp			SE
Soderlind; Erik	Molndal			SE

US-CL-CURRENT: 424/450; 264/4.6, 424/181.1, 424/234.1, 424/422, 424/423, 424/426, 424/448, 424/449, 424/486, 424/499, 424/501, 435/392, 436/174, 436/518, 436/524, 436/527, 436/528, 504/103, 528/272

CLAIMS:

What is claimed is:

1. A method for producing an antigen delivery system comprising a plurality of polymer particles, wherein a water-insoluble protein antigen is incorporated with the polymer particles, the polymer particles comprising a matrix polymer which comprises one or more homo- and/or copolymers, wherein the method comprises: (a) mixing an aqueous phase (W) comprising the water-insoluble protein and one or more hydrophilic surfactants at a concentration of 0.1 to 100 times the critical micelle concentration thereof with an organic phase (O) that comprises the matrix polymer in an organic solvent, which solvent does not denature the protein antigen and wherein O is immiscible with W, to produce a W/O emulsion, wherein either W or O or both further comprise one or more stabilizing agents added prior to mixing to stabilize the W/O emulsion in the presence of the solubilizing agent(s) and promote the incorporation of the water-insoluble protein within the polymer particles during step (b); and (b) forming droplets of said W/O emulsion by dispersing the emulsion in a fluid medium, and removing said solvent from the O phase of the W/O emulsion droplets to thereby form the polymer particles incorporating the water-insoluble protein antigen.

2. The method of claim 1, wherein more than one stabilizing agent is included in the W/O emulsion.

3. The method of claim 2, wherein one of the stabilizing agents is a sorbitan fatty acid ester.

4. The method of claim 2, wherein the stabilizing agents comprise poly (vinyl pyrrolidone) and sodium 1,4-bis(2-ethylhexyl) sulphosuccinate.

5. The method of claim 1 or 2, wherein the one or more stabilizing agents is/are selected from the group consisting of polymers, polar lipids, and hydrophobic surfactants.

6. The method of claim 5, wherein the one or more stabilizing agents is/are a polymer selected from the group consisting of poly(vinyl pyrrolidone), poly (vinyl alcohol), polysaccharides, polyethyleneoxide and water-soluble proteins.

7. The method of claim 5, wherein the one or more stabilizing agents is/are a polar lipid selected from the group consisting of cholesterol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, glycolipids and phosphatidic acid.

8. The method of claim 5, wherein the one or more stabilizing agents is/are a non-ionic, hydrophobic surfactant selected from the group consisting of a sorbitan fatty acid ester, hydrophobic polyoxyethylene alkyl ether, sucrose ester, alkyl-glucopyranoside, polyglycerol polyricinoleate and block-copolymers of ethylene oxide with propyleneoxide and/or lactic acid.

9. The method of claim 5, wherein the one or more stabilizing agents is/are an anionic, hydrophobic surfactant selected from the group consisting of an alkylsulphate salt, a dialkylsulphosuccinate salt, an alkylbenzene sulphonate salt and a fatty acid salt.

10. The method of claim 5, wherein the one or more stabilizing agents is/are a cationic, hydrophobic surfactant selected from the group consisting of an alkyltrimethylammonium salt and a dialkyldimethylammonium salt.

11. The method of claim 1, wherein the aqueous phase comprises more than one solubilizing agent.

12. The method of claim 1, wherein the hydrophilic surfactant is a non-ionic surfactant selected from the group consisting of alkyl-glucopyranosides, alkyl-thioglucopyranosides, alkyl-maltosides, alkoyl-methyl glucamides, glucamides, polyoxyethylene alcohols, polyoxyethylene alkyl phenols, emulphogens, polyoxyethylene sorbitol esters, polyoxyethylene fatty acid esters, hydrophilic polyoxyethylene alkyl ethers and digitonin.

13. The method of claim 1, wherein the hydrophilic surfactant is an anionic surfactant selected from the group consisting of cholates, alkylsulphonates, deoxycholates, alkylsulphates, oligooxyethylene dodecyl ether sulphates and sodium dodecylsarcosinate.

14. The method of claim 1, wherein the hydrophilic surfactant is a cationic surfactant selected from the group consisting of alkylpyridinium salts and alkyltrimethylammonium salts.

15. The method of claim 1, wherein the hydrophilic surfactant is a zwitterionic surfactant selected from the group consisting of 3-1-propanesulphonate (CHAPS), 3-[(3-cholamidopropyl)-dimethylammonio]-2-hydroxy-1-propanesulphonate (CHAPSO), N,N-bis-cholamide (BIGCHAP), N,N-bis-deoxycholamide (deoxy BIGCHAP), lyso phosphatidylcholine, alkylbetaines and sulphobetaines.

16. The method of claim 1 which includes a Double Emulsion (W/O/X) Solvent

Evaporation Technique wherein the fluid medium in which the stabilized W/O emulsion is dispersed in step (b) is a liquid phase (X) which is immiscible with the O phase, said method producing a W/O/X double emulsion comprising W/O droplets from which the solvent is evaporated.

17. The method of claim 1 which includes a Double Emulsion (W/O/X) Solvent Extraction Technique wherein the fluid medium in which the stabilized W/O emulsion is dispersed in step (b) is a liquid phase (X) which is immiscible with the O phase, said method producing a W/O/X double emulsion comprising W/O droplets, and wherein the removal of the organic solvent from the O phase of the droplets is achieved through extraction by the X phase.

18. The method of claim 16 or 17, wherein the X phase comprises a stabilizing agent.

19. The method of claim 18, wherein the one or more stabilizing agents is/are selected from group consisting of polymers, polar lipids, and hydrophobic surfactants.

20. The method of claim 18, wherein the one or more stabilizing agents is/are a polymer selected from the group consisting of poly(vinyl pyrrolidone), poly (vinyl alcohol), polysaccharides, polyethyleneoxide and water soluble proteins.

21. The method of claim 18, wherein the one or more stabilizing agents is/are a polar lipid selected from the group consisting of cholesterol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, glycolipids and phosphatidic acid.

22. The method of claim 18, wherein the one or more stabilizing agents is/are a non-ionic, hydrophobic surfactant selected from the group consisting of sorbitan fatty acid ester, hydrophobic polyoxyethylene alkyl ether, sucrose ester, alkyl-glucopyranoside, polyglycerol polyricinoleate and block-copolymers of ethylene oxide with propyleneoxide and/or lactic acid.

23. The method of claim 18, wherein the one or more stabilizing agents is/are an anionic, hydrophobic surfactant selected from an alkylsulphate salt, dialkylsulphosuccinate salt, alkylbenzene sulphonate salt and a fatty acid salt.

24. The method of claim 18, wherein the one or more stabilizing agents is/are a cationic, hydrophobic surfactant selected from the group consisting of an alkyltrimethylammonium salt and a dialkyldimethylammonium salt.

25. The method of claim 1, wherein the dispersal of the stabilized W/O emulsion in a fluid medium during polymer formulation in step (b) is schieved with a spray drying technique, wherein the stabilized W/O emulsion is dispersed in a gaseous medium to form a spray of W/O emulsion droplets from which said solvent evaporates.

26. The method of claim 1, wherein the dispersal of the stabilized W/O emulsion in a fluid medium during polymer particle formulation in step (b) is achieved with a fluid gas technique.

27. The method of claim 26, wherein the fluid gas technique is selected From the group consisting of gas anti-solvent precipitation (GAS), solution enhanced dispersion by supercritical fluid (SEDS), precipitation with

compressed anti-solvents (PCA), supercritical anti-solvent (SAS) and aerosol solvent extraction system (ASES).

28. The method of claim 1, wherein the protein antigen is a Helicobacter protein or Helicobacter protein fragment.

29. The method of claim 28, wherein the Helicobacter protein or Helicobacter protein fragment is from Helicobacter pylori.

30. The method of claim 28 or 29, wherein said Helicobacter protein is a protein expressed on the surface of Helicobacter.

31. The method of claim 30, wherein the protein part of the lipidated HpaA protein has an amino acid sequence that is identical to, or substantially similar to, positions 28 to 260 of SEQ ID NO. 2 or 4.

32. The method of claim 30, wherein the Helicobacter protein is a lipidated form of Helicobacter pylori adhesion antigen (HpaA).

33. The method of claim 32, wherein the protein is a fully lipidated form of HpaA.

34. The method of claim 1, wherein the matrix polymer is selected from one or more of the group consisting of polyesters, polyanhydrides, polyorthoesters, polycarbonates, polyamides, poly(amino acids), polyacetals, polycyanoacrylates, polyacrylates, biodegradable polyurethanes, non-erodible polyurethanes, polymers of ethylene-vinyl acetate, acyl substituted cellulose acetates, polysaccharides, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolefins, polyethylene oxide, polyethers and polyoxalates.

35. The method of claim 1, wherein the matrix polymer is a polyester homopolymer selected from the group consisting of polylactic acid, polyglycolic acid, polyhydroxybutyrate, poly(alpha hydroxyacids) and polycaprolactone.

36. The method of claim 1, wherein the matrix polymer is a polyester co-polymer selected from the group consisting of poly(lactide-co-glycolide), poly (lactic-co-glycolic acid), poly(hydroxybutyrate-hydroxyvalerate) and poly (lactide-co-caprolactone).

37. The method of claim 36, wherein the matrix polymer is poly(D,L-lactide-co-glycolide).

38. The method according to claim 1 wherein the organic solvent in the organic phase (O) is selected from the group consisting of methylene chloride, chloroform and ethyl acetate.

39. The method of claim 1, wherein in step (a) the W phase is mixed with the O phase in a ratio by volume of 1:10 to 1:1.

40. An antigen delivery system produced by the method of claim 1, wherein the one or more stabilizing agents is/are a polymer selected from the group consisting of poly(vinyl pyrrolidone), poly(vinyl alcohol), polysaccharides, polyethyleneoxide and water soluble proteins, and wherein the method includes a Double Emulsion (W/O/X) Solvent Evaporation Technique wherein the fluid medium in which the stabilized W/O emulsion is dispersed in step (b) is a

liquid phase (X) which is immiscible with the O phase, said method producing a W/O/X double emulsion comprising W/O droplets from which the solvent is evaporated.

41. The antigen delivery system of claim 40, wherein the matrix polymer is selected from one or more of the group consisting of polyesters, polyanhydrides, polyorthoesters, polycarbonates, polyamides, poly(amino acids), polyacetals, polycyanoacrylates, polyacrylates, biodegradable polyurethanes, non-erodible polyurethanes, polymers of ethylene-vinyl acetate, acyl substituted cellulose acetates, polysaccharides, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolefins, polyethylene oxide, polyethers and polyoxalates.

42. The antigen delivery system of claim 41, wherein the polymer is a polyester homopolymer selected from the group consisting of polylactic acid, polyglycolic acid, polyhydroxybutyrate, poly(alpha hydroxyacids) and polycaprolactone.

43. The antigen delivery system of claim 41, wherein the matrix polymer is a polyester co-polymer selected from the group consisting of poly(lactide-co-glycolide), poly(lactic-co-glycolic acid), poly(hydroxybutyrate-hydroxyvalerate) and poly(lactide-co-caprolactone).

44. The antigen delivery system of claim 43, wherein the matrix polymer is poly(D,L-lactide-co-glycolide).

45. The antigen delivery system of any one of claims 40 and 41-44 wherein the polymer particles have an average diameter of 0.05-20 .mu.m according to the volume size distribution.

46. An immunogenic composition comprising the delivery system of claim 45.

47. A method for inducing an immune response directed toward preventing or reducing the risk of Helicobacter infection in a mammalian host, comprising administering to the mammalian host an effective amount of the composition according to claim 46 wherein the water-insoluble protein antigen is a Helicobacter antigen.

48. The method according to claim 47 wherein the protein antigen is a lipidated form of Helicobacter pylori adhesion antigen (HpaA).

49. The method according to claim 48 wherein the protein part of the lipidated antigen has an amino acid sequence that is identical to, or substantially similar to, positions 28 to 260 of SEQ ID NO. 2 or 4.

50. A method for inducing an immune response directed against existing Helicobacter infection in a mammalian host comprising administering to the mammalian host an effective amount of the composition according to claim 46 wherein the water-insoluble protein antigen is a Helicobacter antigen.

51. The method according to claim 50 wherein the protein antigen is a lipidated form of Helicobacter pylori adhesion antigen (HpaA).

52. The method according to claim 51 wherein the protein part of the lipidated antigen has an amino acid sequence that is identical to, or substantially similar to, positions 28 to 260 of SEQ ID NO. 2 or 4.

53. An immunogenic composition comprising the delivery system of any one of claims 40 and 41-44.

54. A method for inducing an immune response directed toward preventing or reducing the risk of Helicobacter infection in a mammalian host, comprising administering to the mammalian host an effective amount of the composition according to claim 53 wherein the water-insoluble protein antigen is a Helicobacter antigen.

55. The method according to claim 54 wherein the protein antigen is a lipidated form of Helicobacter pylori adhesion antigen (HpaA).

56. The method according to claim 55 wherein the protein part of the lipidated antigen has an amino aced sequence that is identical to, or substantially similar to, positions 28 to 260 of SEQ ID NO. 2 or 4.

57. A method for inducing an immune response directed against existing Helicobacter infection in a mammalian host, comprising administering to the mammalian host an effective amount of the composition according to claim 53, wherein the water-insoluble protein antigen is a Helicobacter antigen.

58. The method according to claim 57 wherein the protein antigen is a lipidated form of Helicobacter pylori adhesion antigen (HpaA).

59. The method according to claim 58 wherein the protein part of the lipidated antigen has an amino acid sequence that is identical to, or substantially similar to, positions 28 to 260 of SEQ ID NO. 2 or 4.

60. The composition according to claim 53 wherein the protein antigen is a Helicobacter antigen.

61. The composition according to claim 60 wherein the protein antigen is a lipidated form of Helicobacter pylori adhesion antigen (HpaA).

62. The composition according to claim 61 wherein the protein part of the lipidated antigen has an amino acid sequence that is identical to, or substantially similar to, positions 28 to 260 of SEQ ID NO. 2 or 4.

63. The composition according to claim 46 wherein the protein antigen is a Helicobacter antigen.

64. The composition according to claim 63 wherein the protein antigen is a lipidated form of Helicobacter pylori adhesion antigen (HpaA).

65. The composition according to claim 64 wherein the protein part of the lipidated antigen has an amino acid sequence that is identical to, or substantially similar to, positions 28 to 260 of SEQ ID NO. 2 or 4.

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UniProtKB/Swiss-Prot entry Q9ZJ24

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Entry information

Entry name	**YF88_HELPJ**
Primary accession number	**Q9ZJ24**
Secondary accession numbers	None
Entered in Swiss-Prot in	Release 40, October 2001
Sequence was last modified in	Release 40, October 2001
Annotations were last modified in	Release 44, July 2004

Name and origin of the protein

Protein name	**Hypothetical UPF0174 protein JHP1494**
Synonyms	None
Gene name	OrderedLocusNames: JHP1494
From	Helicobacter pylori J99 (Campylobacter pylori J99) [TaxID: 85963]
Taxonomy	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Helicobacteraceae; Helicobacter.

References

[1] NUCLEOTIDE SEQUENCE [LARGE SCALE GENOMIC DNA].
DOI=10.1038/16495;MEDLINE=99120557;PubMed=9923682 [NCBI, ExPASy, EBI, Israel, Japan]
Alm R.A., Ling L.-S.L., Moir D.T., King B.L., Brown E.D., Doig P.C., Smith D.R., Noonan B., Guild B.C., deJonge B.L., Carmel G., Tummino P.J., Caruso A., Uria-Nickelsen M., Mills D.M., Ives C., Gibson R., Merberg D., Mills S.D., [redacted], Trust T.J.;
"Genomic sequence comparison of two unrelated isolates of the human gastric pathogen Helicobacter pylori.";
Nature 397:176-180(1999).

Comments

- ***SIMILARITY***: Belongs to the UPF0174 family [view classification].

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Cross-references

EMBL	AE001571; AAD07073.1; -.[EMBL / GenBank / DDBJ] [CoDingSequence]
PIR	B71800; B71800.
CMR	Q9ZJ24; JHP1494.

InterPro	IPR005367; UPF0174. Graphical view of domain structure.
Pfam	PF03667; UPF0174; 1. Pfam graphical view of domain structure.
ProDom	[Domain structure / List of seq. sharing at least 1 domain]
HOGENOM	[Family / Alignment / Tree]
BLOCKS	Q9ZJ24.
ProtoNet	Q9ZJ24.
ProtoMap	Q9ZJ24.
PRESAGE	Q9ZJ24.
DIP	Q9ZJ24.
ModBase	Q9ZJ24.
SMR	Q9ZJ24; 127158B2B1A2036A.
SWISS-2DPAGE	Get region on 2D PAGE.
UniRef	View cluster of proteins with at least 50% / 90% identity.

Keywords

Complete proteome; **Hypothetical protein**.

Features

None

Sequence information

Length: **253 AA** Molecular weight: **28476 Da** CRC64: **127158B2B1A2036A** [This is a checksum on the sequence]

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Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter, Dotlet (Java)

ScanProsite, MotifScan

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Swiss-Prot family/domain classification: UPF0174 family

Family/domain
UPF0174 family
Hierarchical classification
all families and domains family **UPF0174 family**
CC SIMILARITY line
This is the list of all Swiss-Prot entries containing the line: `CC -!- SIMILARITY: Belongs to the UPF0174 family.` extracted from the index of CC SIMILARITY lines.
Swiss-Prot entries
Y1587_HELPY (O26106), Y1588_HELPY (O26107), YAAW_ECO57 (P58316), YAAW_ECOLI (P75617), YF87_HELPJ (Q9ZJ25), YF88_HELPJ (Q9ZJ24)

CLUSTAL W (1.74) multiple sequence alignment

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tr|Q5PDN0|Q5PDN0_SALPA      ---LLPLLMKDRSLAKGVSHLLSTQLTRILRTHAAMSILGHGLLRGAG--
tr|O26108|O26108_HELPY      --------------------------------------------------

sp|O26107|Y1588_HELPY       VLTRTLSFLTGPVGWIITGVWTAIDIAGPAYRVTIPACIVVATLRLKTQQ
sp|Q9ZJ24|YF88_HELPJ        VLTRTLSFLTGPVGWIITGVWTAIDIAGPAYRVTIPACIVVATLRLKTQQ
sp|O26106|Y1587_HELPY       ALKKTLGVLAGPIGWVITGALVSINLAGPAYRVTVPACVLIATLRLKLKA
sp|Q9ZJ25|YF87_HELPJ        ALKKTLDILAGPIGWVITGALVSINLAGPAYRVTVPACVLVATLRKKLKA
tr|Q5R096|Q5R096_IDILO      LVTRTIGVALGPVGWFASGLWLAFDLAGPAYRKTIPAVVQIAMLRQLAEK
tr|Q5PDN0|Q5PDN0_SALPA      --------LGGPVGAALNGVKA---MSGSAYRVTIPAVLQIACLRRMMAA
tr|O26108|O26108_HELPY      --------------------------------------------------

sp|O26107|Y1588_HELPY       ANGDKKSLQIESI-------------------------------------
sp|Q9ZJ24|YF88_HELPJ        ANEDKKSLQIESV-------------------------------------
sp|O26106|Y1587_HELPY       K--------------------------------------------------
sp|Q9ZJ25|YF87_HELPJ        E--------------------------------------------------
tr|Q5R096|Q5R096_IDILO      RVNIGIVGEGSCGKDSLIRETFGVDTNNVSAVPGSTSKAEAYALNEAATV
tr|Q5PDN0|Q5PDN0_SALPA      VQA----------------------------------------------
tr|O26108|O26108_HELPY      --------------------------------------------------

sp|O26107|Y1588_HELPY       --------------------------------------------------
sp|Q9ZJ24|YF88_HELPJ        --------------------------------------------------
sp|O26106|Y1587_HELPY       --------------------------------------------------
```

```
sp|Q9ZJ25|YF87_HELPJ        --------------------------------------------------
tr|Q5R096|Q5R096_IDILO      MNYAGFHDSEHEVNENTADYLIHTDVFVWVVDIQRGITGTELETFEKLKR
tr|Q5PDN0|Q5PDN0_SALPA      --------------------------------------------------
tr|O26108|O26108_HELPY      --------------------------------------------------

sp|O26107|Y1588_HELPY       --------------------------------------------------
sp|Q9ZJ24|YF88_HELPJ        --------------------------------------------------
sp|O26106|Y1587_HELPY       --------------------------------------------------
sp|Q9ZJ25|YF87_HELPJ        --------------------------------------------------
tr|Q5R096|Q5R096_IDILO      YNRPVVLCINKVDTPKNDADKEALINSINERLELNSGKSSLIKAVFETAF
tr|Q5PDN0|Q5PDN0_SALPA      --------------------------------------------------
tr|O26108|O26108_HELPY      --------------------------------------------------

sp|O26107|Y1588_HELPY       ------------------------------------
sp|Q9ZJ24|YF88_HELPJ        ------------------------------------
sp|O26106|Y1587_HELPY       ------------------------------------
sp|Q9ZJ25|YF87_HELPJ        ------------------------------------
tr|Q5R096|Q5R096_IDILO      DPDPRLMEKAIGGDEVLGFLRNFLSEKLGKDSDCLDLA
tr|Q5PDN0|Q5PDN0_SALPA      ------------------------------------
tr|O26108|O26108_HELPY      ------------------------------------
```

PileUp

MSF: 438 Type: P Check: 4658 ..

Name: sp|O26107|Y1588_HELPY oo Len: 438 Check: 3827 Weight: 0.100
Name: sp|Q9ZJ24|YF88_HELPJ oo Len: 438 Check: 4232 Weight: 0.100
Name: sp|O26106|Y1587_HELPY oo Len: 438 Check: 2786 Weight: 0.100
Name: sp|Q9ZJ25|YF87_HELPJ oo Len: 438 Check: 1811 Weight: 0.100
Name: tr|Q5R096|Q5R096_IDILO oo Len: 438 Check: 7769 Weight: 0.100
Name: tr|Q5PDN0|Q5PDN0_SALPA oo Len: 438 Check: 905 Weight: 0.100
Name: tr|O26108|O26108_HELPY oo Len: 438 Check: 3328 Weight: 0.100

//

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sp|O26107|Y1588_HELPY       ..MAYKYDRD LEFLKQLESS DLLDLFEVLV FGKDGEKRHN EKLTS...SI
sp|Q9ZJ24|YF88_HELPJ        ..MAYKYDRD LEFLKQLESS DLLDLFEVLV FGKDGEKRHN EKLTS...SI
sp|O26106|Y1587_HELPY       .......... .......MNE DLTNSTEYKR YGHDYAKYPR R.........
sp|Q9ZJ25|YF87_HELPJ        .......... .......MNE ELTSLTEYQR YGHDYAKYPR R.........
tr|Q5R096|Q5R096_IDILO      .....MNNHP VETLCKTHYA DILPLVEYLK VDKDLQRSIG IAAREAQQQT
tr|Q5PDN0|Q5PDN0_SALPA      MNVTYLHDED LDFLQHCSEE QLADFARLLT HNEKGKARLS SVLSHNELFK
tr|O26108|O26108_HELPY      ..MAYRYDSD LEFLKRLSSS DLKDLFDALV YDEDGTLRMN E.........

sp|O26107|Y1588_HELPY       EYKRHGDDYA KYAERIAEEL QYYGSNSFAS FIKGEGVLYK EILCDVCDKL
sp|Q9ZJ24|YF88_HELPJ        EYKRHGDDYA KYAERIAEEL QYYGSNSFAS FIKGEGVLYK EILCDVCDKL
sp|O26106|Y1587_HELPY       .......... .....IAEEL QHYGGNSFAN FFRDEGVLYK EILCDACDHL
sp|Q9ZJ25|YF87_HELPJ        .......... .....IAEEL QRYGGNSFAN FFRDEGVLYK EILCDACDHL
tr|Q5R096|Q5R096_IDILO      GNTANYFVKE QHAEQLINDL RDAGSNSLKS VFT.EPSYYS EIVYDVGLKL
tr|Q5PDN0|Q5PDN0_SALPA      AMEGHPEQHR RNWQLIAGEF QHYGGDSIAN KLRGHGKQYR AILLDVAKRL
tr|O26108|O26108_HELPY      .......... .......... .......... .......... ..........

sp|O26107|Y1588_HELPY       KVNYNKKTET TLIEQNMLSK ILERSLEEMD DEEVKEMCDE LSIKNTDNLN
sp|Q9ZJ24|YF88_HELPJ        KVNYNKKTET TLIEQNMLSK ILERSLEEMD DEEVKEMCDE LSIKNTDNLN
sp|O26106|Y1587_HELPY       KVNYNEESAT SLIEQNMLSK LLKDSLEKMS RREIKELCNE LGMTNIDKVI
sp|Q9ZJ25|YF87_HELPJ        DINYNERSAT SLIEQNMLSK LLKDSLEKMS GREIKELCDG LGMPNIDKVI
tr|Q5R096|Q5R096_IDILO      KADVSKTNLA KENEDLIIGK LFADAVAEMS EEEKSELLLE FGYETTKIPA
tr|Q5PDN0|Q5PDN0_SALPA      KLKADKSMST FEIEQQLLEH FLRHTWQKMD AAHKQEFLQA VDAKVSELEE
tr|O26108|O26108_HELPY      .......... .......... .......... .......... ..........

sp|O26107|Y1588_HELPY       ...RQALSAA TLTLFK.MGG FKSYQLAVIV ANAVAKTILG RGLS.LAGNQ
sp|Q9ZJ24|YF88_HELPJ        ...RQALSAA TLTLFK.MGG FKSYQLAVIV ANAVAKTILG RGLS.LAGNQ
sp|O26106|Y1587_HELPY       GENKQVLIAS TLTLFK.AGG SHSYALAVSV ADAMVRQTLG HXACYVVGKV
sp|Q9ZJ25|YF87_HELPJ        GENKQVLIAS VLTLFK.AGG SHSYALAVAV ADAMVRQTLG HGLSSVVGKV
tr|Q5R096|Q5R096_IDILO      ...ALSVMGT QLGLRS..LG FSTYRMAVII ANYIARALLN RGLT.FGGNI
tr|Q5PDN0|Q5PDN0_SALPA      ...LLPLLMK DRSLAKGVSH LLSTQLTRIL RTHAAMSILG HGLLRGAG..
tr|O26108|O26108_HELPY      .......... .......... .......... .......... ..........

sp|O26107|Y1588_HELPY       VLTRTLSFLT GPVGWIITGV WTAIDIAGPA YRVTIPACIV VATLRLKTQQ
sp|Q9ZJ24|YF88_HELPJ        VLTRTLSFLT GPVGWIITGV WTAIDIAGPA YRVTIPACIV VATLRLKTQQ
sp|O26106|Y1587_HELPY       ALKKTLGVLA GPIGWVITGA LVSINLAGPA YRVTVPACVL IATLRLKLKA
sp|Q9ZJ25|YF87_HELPJ        ALKKTLDILA GPIGWVITGA LVSINLAGPA YRVTVPACVL VATLRKKLKA
tr|Q5R096|Q5R096_IDILO      LVTRTIGVAL GPVGWFASGL WLAFDLAGPA YRKTIPAVVQ IAMLRQLAEK
tr|Q5PDN0|Q5PDN0_SALPA      ........LG GPVGAALNGV KA...MSGSA YRVTIPAVLQ IACLRRMMAA
```

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tr|O26108|O26108_HELPY      .......... .......... .......... .......... ..........


sp|O26107|Y1588_HELPY       ANGDKKSLQI ESI....... .......... .......... ..........
sp|Q9ZJ24|YF88_HELPJ        ANEDKKSLQI ESV....... .......... .......... ..........
sp|O26106|Y1587_HELPY       K......... .......... .......... .......... ..........
sp|Q9ZJ25|YF87_HELPJ        E......... .......... .......... .......... ..........
tr|Q5R096|Q5R096_IDILO      RVNIGIVGEG SCGKDSLIRE TFGVDTNNVS AVPGSTSKAE AYALNEAATV
tr|Q5PDN0|Q5PDN0_SALPA      VQA....... .......... .......... .......... ..........
tr|O26108|O26108_HELPY      .......... .......... .......... .......... ..........


sp|O26107|Y1588_HELPY       .......... .......... .......... .......... ..........
sp|Q9ZJ24|YF88_HELPJ        .......... .......... .......... .......... ..........
sp|O26106|Y1587_HELPY       .......... .......... .......... .......... ..........
sp|Q9ZJ25|YF87_HELPJ        .......... .......... .......... .......... ..........
tr|Q5R096|Q5R096_IDILO      MNYAGFHDSE HEVNENTADY LIHTDVFVWV VDIQRGITGT ELETFEKLKR
tr|Q5PDN0|Q5PDN0_SALPA      .......... .......... .......... .......... ..........
tr|O26108|O26108_HELPY      .......... .......... .......... .......... ..........


sp|O26107|Y1588_HELPY       .......... .......... .......... .......... ..........
sp|Q9ZJ24|YF88_HELPJ        .......... .......... .......... .......... ..........
sp|O26106|Y1587_HELPY       .......... .......... .......... .......... ..........
sp|Q9ZJ25|YF87_HELPJ        .......... .......... .......... .......... ..........
tr|Q5R096|Q5R096_IDILO      YNRPVVLCIN KVDTPKNDAD KEALINSINE RLELNSGKSS LIKAVFETAF
tr|Q5PDN0|Q5PDN0_SALPA      .......... .......... .......... .......... ..........
tr|O26108|O26108_HELPY      .......... .......... .......... .......... ..........


sp|O26107|Y1588_HELPY       .......... .......... .......... ........
sp|Q9ZJ24|YF88_HELPJ        .......... .......... .......... ........
sp|O26106|Y1587_HELPY       .......... .......... .......... ........
sp|Q9ZJ25|YF87_HELPJ        .......... .......... .......... ........
tr|Q5R096|Q5R096_IDILO      DPDPRLMEKA IGGDEVLGFL RNFLSEKLGK DSDCLDLA
tr|Q5PDN0|Q5PDN0_SALPA      .......... .......... .......... ........
tr|O26108|O26108_HELPY      .......... .......... .......... ........
```

CLUSTAL W (1.74) multiple sequence alignment

```
sp|O26107|Y1588_HELPY       --MAYKYDRDLEFLKQLESSDLLDLFEVLVFGKDGEKRHNEKLTS---SI
sp|Q9ZJ24|YF88_HELPJ        --MAYKYDRDLEFLKQLESSDLLDLFEVLVFGKDGEKRHNEKLTS---SI
tr|Q5PDN0|Q5PDN0_SALPA      MNVTYLHDEDLDFLQHCSEEQLADFARLLTHNEKGKARLSSVLSHNELFK
                              ::* :*.**:**:: ...:* *: .:*...:.*: * .. *:

sp|O26107|Y1588_HELPY       EYKRHGDDYAKYAERIAEELQYYGSNSFASFIKGEGVLYKEILCDVCDKL
sp|Q9ZJ24|YF88_HELPJ        EYKRHGDDYAKYAERIAEELQYYGSNSFASFIKGEGVLYKEILCDVCDKL
tr|Q5PDN0|Q5PDN0_SALPA      AMEGHPEQHRRNWQLIAGEFQHYGGDSIANKLRGHGKQYRAILLDVAKRL
                              : * ::: :  : ** *:*:**.:*:*. ::*.*  *: ** **..:*

sp|O26107|Y1588_HELPY       KVNYNKKTETTLIEQNMLSKILERSLEEMDDEEVKEMCDELSIKNTDNLN
sp|Q9ZJ24|YF88_HELPJ        KVNYNKKTETTLIEQNMLSKILERSLEEMDDEEVKEMCDELSIKNTDNLN
tr|Q5PDN0|Q5PDN0_SALPA      KLKADKSMSTFEIEQQLLEHFLRHTWQKMDAAHKQEFLQAVDAKVSELEE
                            *:: :*. .*  ***::*.::*.:: ::**  . :*: : :. * ::  :

sp|O26107|Y1588_HELPY       RQALSAATLTLFK-MGGFKSYQLAVIVANAVAKTILGRGLSLAGNQVLTR
sp|Q9ZJ24|YF88_HELPJ        RQALSAATLTLFK-MGGFKSYQLAVIVANAVAKTILGRGLSLAGNQVLTR
tr|Q5PDN0|Q5PDN0_SALPA      LLPLLMKDRSLAKGVSHLLSTQLTRILRTHAAMSILGHGL-LRG--AG--
                              .*     :* * :. : * **: *: . .* :***:** * *  .

sp|O26107|Y1588_HELPY       TLSFLTGPVGWIITGVWTAIDIAGPAYRVTIPACIVVATLRLKTQQANGD
sp|Q9ZJ24|YF88_HELPJ        TLSFLTGPVGWIITGVWTAIDIAGPAYRVTIPACIVVATLRLKTQQANED
tr|Q5PDN0|Q5PDN0_SALPA      ----LGGPVGAALNGVKAMS---GSAYRVTIPAVLQIACLRRMMAAVQA-
                                * ****  :.** :     *.******** : :* **     .:

sp|O26107|Y1588_HELPY       KKSLQIESI
sp|Q9ZJ24|YF88_HELPJ        KKSLQIESV
tr|Q5PDN0|Q5PDN0_SALPA      ---------
```

PileUp

MSF: 259 Type: P Check: 3539 ..

Name: sp|O26107|Y1588_HELPY oo Len: 259 Check: 4393 Weight: 0.100
Name: sp|Q9ZJ24|YF88_HELPJ oo Len: 259 Check: 4754 Weight: 0.100
Name: tr|Q5PDN0|Q5PDN0_SALPA oo Len: 259 Check: 4392 Weight: 0.100

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sp|O26107|Y1588_HELPY       ..MAYKYDRD LEFLKQLESS DLLDLFEVLV FGKDGEKRHN EKLTS...SI
sp|Q9ZJ24|YF88_HELPJ        ..MAYKYDRD LEFLKQLESS DLLDLFEVLV FGKDGEKRHN EKLTS...SI
tr|Q5PDN0|Q5PDN0_SALPA      MNVTYLHDED LDFLQHCSEE QLADFARLLT HNEKGKARLS SVLSHNELFK

sp|O26107|Y1588_HELPY       EYKRHGDDYA KYAERIAEEL QYYGSNSFAS FIKGEGVLYK EILCDVCDKL
sp|Q9ZJ24|YF88_HELPJ        EYKRHGDDYA KYAERIAEEL QYYGSNSFAS FIKGEGVLYK EILCDVCDKL
tr|Q5PDN0|Q5PDN0_SALPA      AMEGHPEQHR RNWQLIAGEF QHYGGDSIAN KLRGHGKQYR AILLDVAKRL

sp|O26107|Y1588_HELPY       KVNYNKKTET TLIEQNMLSK ILERSLEEMD DEEVKEMCDE LSIKNTDNLN
sp|Q9ZJ24|YF88_HELPJ        KVNYNKKTET TLIEQNMLSK ILERSLEEMD DEEVKEMCDE LSIKNTDNLN
tr|Q5PDN0|Q5PDN0_SALPA      KLKADKSMST FEIEQQLLEH FLRHTWQKMD AAHKQEFLQA VDAKVSELEE

sp|O26107|Y1588_HELPY       RQALSAATLT LFK.MGGFKS YQLAVIVANA VAKTILGRGL SLAGNQVLTR
sp|Q9ZJ24|YF88_HELPJ        RQALSAATLT LFK.MGGFKS YQLAVIVANA VAKTILGRGL SLAGNQVLTR
tr|Q5PDN0|Q5PDN0_SALPA      LLPLLMKDRS LAKGVSHLLS TQLTRILRTH AAMSILGHGL .LRG..AG..

sp|O26107|Y1588_HELPY       TLSFLTGPVG WIITGVWTAI DIAGPAYRVT IPACIVVATL RLKTQQANGD
sp|Q9ZJ24|YF88_HELPJ        TLSFLTGPVG WIITGVWTAI DIAGPAYRVT IPACIVVATL RLKTQQANED
tr|Q5PDN0|Q5PDN0_SALPA      ....LGGPVG AALNGVKAMS ...GSAYRVT IPAVLQIACL RRMMAAVQA.

sp|O26107|Y1588_HELPY       KKSLQIESI
sp|Q9ZJ24|YF88_HELPJ        KKSLQIESV
tr|Q5PDN0|Q5PDN0_SALPA      .........
```

Summary of Invention Paragraph:
[0007] Monoclonal antibodies (MAbs) against membrane preparations of H. pylori have been disclosed by Bolin et al. (1995) J. Clin. Microbiol. 33, 381-384. One of these MAbs, designated HP30-1:1:6, reacted with a 30 kDa protein which was shown to be exposed on the surface of intact bacteria and to have properties like that of an adhesin.

European Patent Office

SUPPLEMENTARY PARTIAL EUROPEAN SEARCH REPORT

under Rule 46, paragraph 1 of the European Patent Convention

Application Number EP 01 99 4245

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	WO 97/19098 A (ASTRA AB ; SMITH DOUGLAS H (US)) 29 May 1997 (1997-05-29) SEQ ID NO:250 and SEQ ID NO:91 * the whole document *	1-5, 7-21,25, 26	C12N15/31

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

C12N
C07K
A61K

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

1 The present partial European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims.

Place of search	Date of completion of the search	Examiner
Munich	29 October 2004	Herrmann, K

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C23)

European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 01 99 4245

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. claims: 1-26 (all partially)

Polypeptide consisting of the amino acid sequence as in SEQ ID NO:4 or SEQ ID NO:48 ("HP30") and subject-matter relating thereto. Polynucleotides encoding the polypeptide of SEQ ID NO:4 or SEQ ID NO:48 (98.8% identical) such as a polynucleotide according to SEQ ID NO:3 or 47, respectively, and subject-matter relating thereto.

---

2. claims: 1-26 (all partially)

Polypeptide consisting of the amino acid sequence as in SEQ ID NO:2 ("HP56") and subject-matter relating thereto. Polynucleotides encoding the polypeptide of SEQ ID NO:2 such as a polynucleotide according to SEQ ID NO:1 and subject-matter relating thereto.

European Patent Office

INCOMPLETE SEARCH SHEET C

Application Number
EP 01 99 4245

Claim(s) searched completely:
1-5, 7-21, 25, 26

Claim(s) not searched:
6, 22-24

Reason for the limitation of the search:

Claims 6 and 22:
Claims 6 and 22 fail to comply with the requirements of Art. 84 PCT (clarity) to such an extent that a meaningful search could not be carried out (Guidelines B-III, 3.12). Claim 6 refers to claim 63, claim 22 refers to claim 56. However, present set of claims contains 26 claims only.

Claims 23 and 24:
Compounds as such are not sufficiently defined by their mode of action. Therefore, claims 23 and 24 have not been searched because antagonists are neither disclosed nor supported within the terms of Art. 83 and 84 EPC, respectively.

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 01 99 4245

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

29-10-2004

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9719098 A	29-05-1997	AU 1055497 A	11-06-1997
		NO 975745 A	09-02-1998
		SK 165197 A3	11-01-1999
		US 2003019938 A1	30-01-2003
		WO 9640893 A1	19-12-1996
		WO 9719098 A1	29-05-1997
		US 2002185542 A1	12-12-2002
		US 6595420 B1	22-07-2003

EPO FORM P0450

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

REMARKS

Claims 1 - 78 are required to be restricted to one of 19 different groups; i.e. Groups I - XIX (Office Action at pages 2 to 5).

In response, Applicants elect, with traverse, to prosecute the subject matter of Group I, claims 1 - 7, 8 - 10, 15 - 24, 25 - 29, 41, 57 - 59, directed to an isolated Helicobacter species polypeptide of about 30 KDa, fragments, fusion polypeptides, and compositions comprising the same, as well as claims 42 - 44, 60 - 62, 67, 68, 69 directed to methods of using the same.

Further, as indicated at pages 8 - 9 of the Office Action, the claims of Group I are stated to be directed to the following "patentably distinct species" and election of a single species is required:

Group I species:

a) 30 KDa polypeptide;

b) fragments of 30 KDa of at least 6 amino acids of SEQ ID No. 4;

c) fusion protein of two SEQ ID Nos. selected from SEQ ID Nos. 16 - 20;

d) fusion protein of three SEQ ID Nos. selected from SEQ ID Nos. 16 - 20;

e) fusion protein of four SEQ ID Nos. selected from SEQ ID Nos. 16 - 20;

and

f) fusion protein of five SEQ ID Nos. selected from SEQ ID Nos. 16 - 20.

In reply, Applicants elect the species: a, i.e., 30 KDa polypeptide.

Claims 1-5, 15-19, 41-42, 57-59, 60, 67, 68 and 69 to the extent limited to Group I, species a read on the elected Group I and species.

Applicants understand that, upon allowance of a generic claim, Applicants will be entitled to consideration of claims to additional species written in dependent form. It is noted that the Office Action indicated that none of the original claims to separate species were generic. Accordingly, and in accord with the elections above, claims 1-7, 8-10, 15-24, 25-29, 41, 42-44, 57-59, 60-62, and 67-69 are amended herein to be generic to the specie identified and to be directed to the subject matter of elected Group I. No new matter is added and all the claims are fully supported by the specification and claims as originally filed.